

Listing of Claims:

1 (original) A live attenuated derivative of a pathogenic *Salmonella* species consisting essentially of

(a) a means for regulatable expression of a gene that encodes a regulatory protein, wherein non-expression of said regulatory protein in vivo causes synthesis of a first antigen that is conserved among *Salmonella* species and *E. coli* strains; and

(b) a means for regulatable synthesis of a first carbohydrate antigen, wherein said first carbohydrate antigen ceases to be synthesized in vivo, exposing a second carbohydrate antigen that is conserved among *Salmonella* species and *E. coli* strains;

wherein said attenuated derivative has enhanced ability to induce cross-protective immunity against *Salmonella* species and *E. coli* strains.

2 (original) The live attenuated derivative of claim 1, further comprising a means for non-expression of a serotype-specific antigen.

3 (original) The live attenuated derivative of claim 2, wherein said means for non-expression of a serotype-specific antigen comprises a mutation in a gene selected from the group consisting of *fliC* and *fliB*.

4 (original) The live attenuated derivative of claim 3, wherein said mutation is a deletion mutation.

5 (original) The live attenuated derivative of claim 1, wherein said means of regulatable expression comprises substituting the promoter of said gene that encodes a regulatory protein with a regulatable promoter.

6 (original) The live attenuated derivative of claim 5 wherein said regulatable promoter is the *araCP_{BAD}* repressor-activator-promoter system.

7 (original) The live attenuated derivative of claim 6 wherein said gene that encodes a regulatory protein is selected from the group consisting of *fur*, *rpoS*, *phoPQ*, *dam*, *ompR*, *cya* and *crp*.

8 (original) The live attenuated derivative of claim 1 wherein said carbohydrate antigen is an LPS O-antigen.

9 (original) The live attenuated derivative of claim 8 wherein said means for regulatable synthesis comprises a mutation in a gene that encodes a product necessary for synthesis of LPS O-antigen.

10 (original) The live attenuated derivative of claim 9, wherein said means for regulatable synthesis comprises a mutation in the *pmi* gene.

11 (original) A method for inducing an immune response sufficient for protection against infection by *Salmonella* species and *E. coli* strains, said method comprising administering to an individual the live attenuated derivative of claim 1.

12 (original) A live attenuated derivative of a pathogenic *Salmonella* species, consisting essentially of

(a) a means for regulatable expression of a *fur* gene; and

(b) a mutation that renders a *pmi* gene inoperable,
wherein said attenuated derivative has enhanced ability to induce cross-protective immunity against *Salmonella* species and *E. coli*.

13 (original) The live attenuated derivative of claim 12 wherein said means of (a) comprises substituting the *fur* promoter with a regulatable promoter.

14 (original) The live attenuated derivative of claim 12, wherein said means of (a) comprises replacing the *fur* promoter with the *araCP_{BAD}* activator-repressor-promoter system.

15 (original) The live attenuated derivative of claim 12 wherein said means of (a) comprises the $\Delta P_{fur}::araCP_{BAD}fur$ genetic construction.

16 (original) The live attenuated derivative of claim 12 wherein said mutation of (b) is a deletion mutation.

17 (currently amended) A method of inducing a cross-protective immune response against *Salmonella* species, said method comprising administering to an individual the live attenuated derivative of any of claims 2-4.

18 (original) A live attenuated derivative of a pathogenic *Salmonella* species consisting essentially of

(a) a means for regulatable expression of a first surface antigen, wherein said first surface antigen is conserved among *Salmonella* species and *E. coli* strains; and

(b) a means for regulatable expression of a second surface antigen, wherein said second surface antigen is not conserved among *Salmonella* species and *E. coli* strains,

wherein up regulation of said first surface antigen and down regulation of said second surface antigen results in enhanced ability of said attenuated derivative to produce immunity against *Salmonella* species and *E. coli* strains.

19 (original) A vaccine comprising a live attenuated strain of *Salmonella*, wherein said live attenuated strain consists essentially of

(a) a mutation in a *pmi* gene that renders said *pmi* gene non functional; and;

(b) a genetic construction that allows for regulatable expression of a *fur* gene,

wherein said vaccine has enhanced ability to stimulate cross protective immunity against *Salmonella* species and *E. coli* strains.

20 (original) A method for inducing an immune response to *Salmonella* species and *E. coli* strains comprising administering to an individual a live attenuated derivative of a pathogenic *Salmonella* species that is capable of colonizing the intestinal tract and reaching and persisting in the Gut Associated Lymphoid Tissue, and wherein expression of at least one conserved surface antigen is up regulated and at least one non-conserved surface antigen is down regulated in said attenuated derivative when said attenuated derivative is in the lymphoid tissue of the individual, wherein said live attenuated derivative has enhanced ability to stimulate cross protective immunity against infection by *Salmonella* species and *E. coli* strains.

21 (original) A vaccine comprising a live attenuated strain of *Salmonella*, wherein said live attenuated strain consists essentially of

- (a) a mutation that renders a *pmi* gene non functional; and
- (b) a regulatable promotor operably linked to a *fur* gene wherein said *fur* gene is expressed when said attenuated strain is in the intestinal tract of an individual and said *fur* gene is not expressed when said attenuated strain is within internal tissues of an individual.

22 (original) The vaccine of claim 21 wherein said regulatable promoter comprises the *araCP_{BAD}* activator-repressor-promoter system.

23 (original) A live attenuated derivative of a *Salmonella* species consisting essentially of

- (a) a means for regulatable synthesis of LPS O-antigen side chains, wherein said O-antigen side chains are synthesized when said attenuated derivative is in the intestinal tract of an individual and are not synthesized when said attenuated derivative is within internal tissues of an individual; and
- (b) a means for regulatable expression of a *fur* gene, wherein said *fur* gene is expressed when said attenuated derivative is in the intestinal tract of an individual and wherein said *fur* gene is not expressed when said attenuated derivative within internal tissues of an individual

wherein said attenuated derivative has increased ability to induce cross-protective immunity against infection by *Salmonella* species and *E. coli* strains.

24 (original) The live attenuated derivative of claim 23 wherein said means for regulatable synthesis comprises a mutation in a gene that encodes a product necessary for synthesis of LPS O-antigens.

25 (original) The live attenuated derivative of claim 24 wherein said gene that encodes a product necessary for synthesis of LPS O-antigens is a *pmi* gene.

26 (original) A live attenuated derivative of a *Salmonella typhimurium* comprising
(a) a $\Delta P_{fur}::T\lambda aracP_{BAD}fur$ deletion-insertion mutation; and
(b) a Δpmi mutation

27 (original) A recombinant bacterial strain consisting essentially of a means of regulatable expression of a virulence gene, wherein said regulatable expression of a virulence gene renders said bacterial strain attenuated while maintaining immunogenicity.

28 (original) The recombinant *Salmonella* of claim 27, wherein said virulence gene is selected from the group consisting of *aroA*, *aroC*, *aroD*, *cya*, *crp*, *cdt*, *ompR*, *htrA*, *hemA*, *purA*, *purB*, *rfa*, *rbf*, *asd* *ompC* and *ompF*.

29 (original) The recombinant bacterial strain of claim 27, wherein said means of regulatable expression comprises substituting the promoter for said virulence gene with the *araCP_{BAD}* repressor-activator-promoter system.

30 (original) The recombinant bacterial strain of claim 29, wherein said virulence gene is a *fur* gene.

31 (original) The recombinant bacterial strain of claim 30, further comprising a Δpmi mutation.

32 (original) A live attenuated derivative of a pathogenic *Enterobacteriaceae* species consisting essentially of a $\Delta P_{fur}::araCP_{BAD}fur$ genetic construction.

33 (original) A live attenuated derivative of a pathogenic *Salmonella* species consisting essentially of

(a) a means for regulatable expression of a gene that encodes a regulatory protein, wherein non-expression of said regulatory protein in vivo causes synthesis of a first antigen that is conserved among *Salmonella* species and *E. coli* strains; and

(b) a means for regulatable synthesis of a first carbohydrate antigen, wherein said first carbohydrate antigen ceases to be synthesized in vivo, exposing a second carbohydrate antigen that is conserved among *Salmonella* species and *E. coli* strains; and

(c) a mutation of *fliC* or *fliB*, wherein said mutation results in deletion of the variable domain while retaining the N-terminal and C-terminal constant domains of flagellar proteins;

wherein said attenuated derivative has enhanced ability to induce cross-protective immunity against *Salmonella* species and *E. coli* strains.

34 (original) The live attenuated derivative of claim 1, further comprising a means for biological containment.

35 (original) The live attenuated derivative of claim 34, wherein said means comprises a mutation that abolishes motility, prevents synthesis of the exopolysaccharide colanic acid, prevents synthesis of components of the bacterial extracellular matrix, reduces ability to withstand the stresses of stationary phase and starvation, reduces ability to use nucleic acids as a nutrient, or uncouples regulation of cellular activities from a dependence on protein synthesis.

36 (original) The live attenuated derivative of claim 35, wherein said mutation is selected from the group consisting of $\Delta(gmd-fcl)$ -26, $\Delta agfBAC811$, $\Delta bcsABZC2118$, $\Delta bcsABZC2119$, $\Delta adrA1418$, $\Delta mlrA34$, $\Delta yhiR36::TT$, $\Delta endA2311$, $\Delta relA1123$.

37 (original) The live attenuated derivative of claim 35, wherein said mutation consists of a mutation in a gene selected from the group consisting of *gmd*, *fcl*, *agf*, *bcs*, *adr*, *mlr*, *yhi*, *end* and *rel*.

38 (original) The live attenuated derivative of claim 1, further comprising a mutation in a gene selected from the group consisting of *sip* and *sop*.

39 (original) The live attenuated derivative of claim 38, wherein said mutation is $\Delta sopB1925$.

40 (original) The live attenuated derivative of claim 1, wherein said live attenuated derivative comprises the $\Delta ilvG3::TTaraCP_{BAD}/lacI$ genetic construction.